Biochemistry 461, Section I Your Printed Name:_____

May 6, 1997

Exam #3 Your SS#:_____

Prof. Jason D. Kahn

Your Signature:_____

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Some information which may be useful is provided on the bottom half of this page.

Explanations should be concise and answer the specific question asked. You have more space on the exam than is necessary—don't think you need to fill the page.

You will not need a calculator for this exam. No other study aids or materials are permitted.

Possibly Useful Information:

Michaelis-Menten equation: $v_0 = V_{max}[S]/(K_m + [S])$

Type of inhibition	Apparent K_m	Apparent V_{max}	Apparent V_{max}/K_m
Competitive	αK_m	V_{max}	$(1/\alpha)\ V_{max}/K_m$
Uncompetitive	$(1/\alpha')K_m$	$(1/\alpha')V_{max}$	V_{max}/K_m
Mixed	$(\alpha/\alpha')K_m$	$(1/\alpha')V_{max}$	$(1/\alpha)\ V_{max}/K_m$
Noncompetitive ($\alpha=\alpha'$)	K_m	$(1/\alpha)\ V_{max}$	$(1/\alpha)\ V_{max}/K_m$

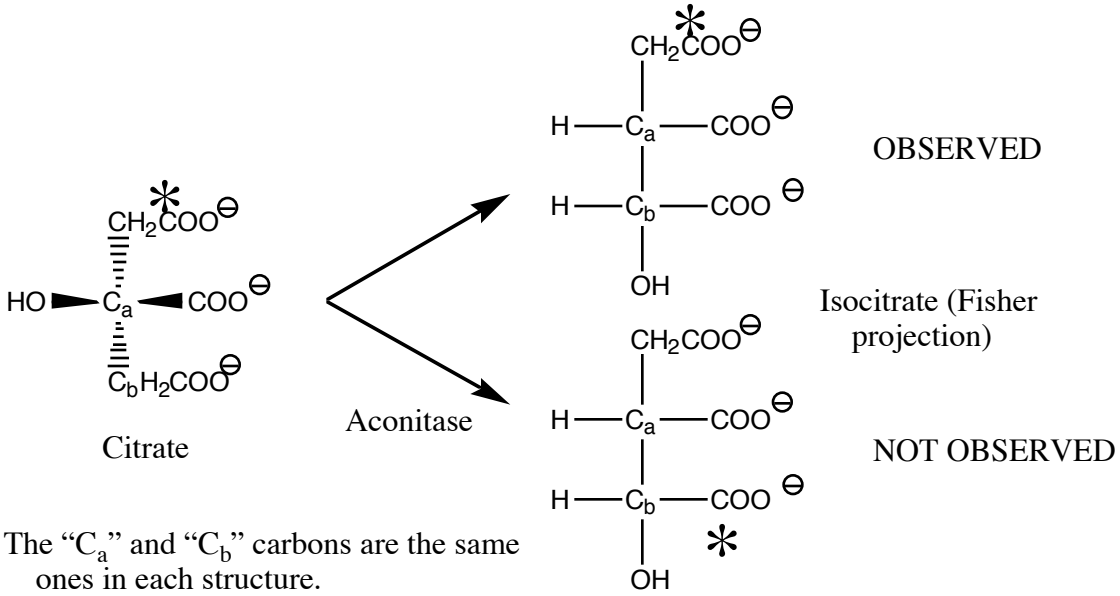
$\alpha = 1 + [I]/K_I$ $\alpha' = 1 + [I]/K_{I'}$

1. (20 pts) Fundamentals of Enzymatic Catalysis.

(a; 8 pts) List four types of enzymatic catalysis and give very brief descriptions for two of these.

(b; 4 pts) Why are enzyme active sites typically in pockets or clefts on the enzyme surface?

(c; 8 pts) Citrate was proposed in the 1930's to be a critical intermediate in the Krebs cycle, which is central to metabolism. Citrate is achiral, as two substituents (the CH_2COO^- groups) of the central carbon atom are the same. It is converted to isocitrate as shown, by the enzyme aconitase. Later investigators (1940's) were surprised to find that an isotopic label on citrate (with the label indicated by the star) ended up in only one of the two possible places in isocitrate. Why did they initially, incorrectly, expect that label should go to both of the indicated positions? Propose a simple explanation for the observed specificity and draw a picture to illustrate your answer. The answer has to do only with the binding of citrate to the enzyme surface, not with the mechanism of aconitase. Hint: if the reaction were just acid or base-catalyzed, both products would be observed.

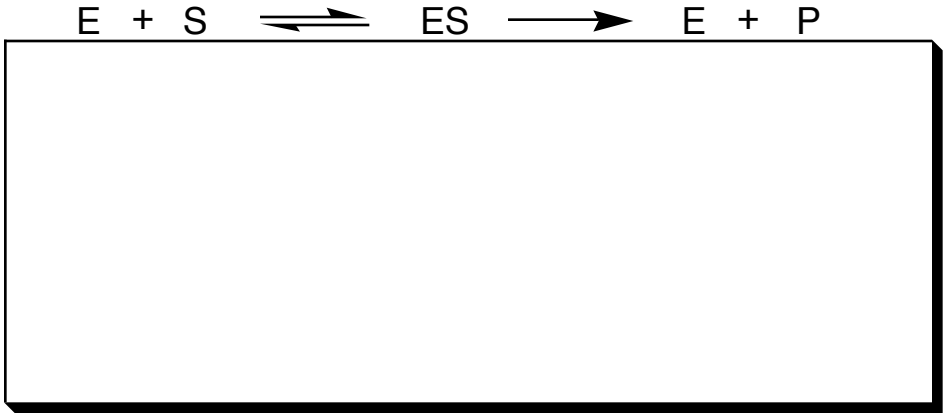


2. (25 points) Enzyme Kinetics.

(a; 7 pts) Given the Michaelis-Menten equation on the front page, derive the double-reciprocal (Lineweaver-Burk) expressions used to linearize v_0 vs. [S] data, and sketch the resulting plot. Label the axes, the slope, and the *x* and *y* intercepts.

(b; 5 pts) What is the operational definition of K_m (i.e. how is it measured)? What is K_m in terms of the three elementary rate constants of the Michaelis-Menten equation? Why is it that measuring a K_m is useful even when the actual mechanism is more complicated than the M-M mechanism?

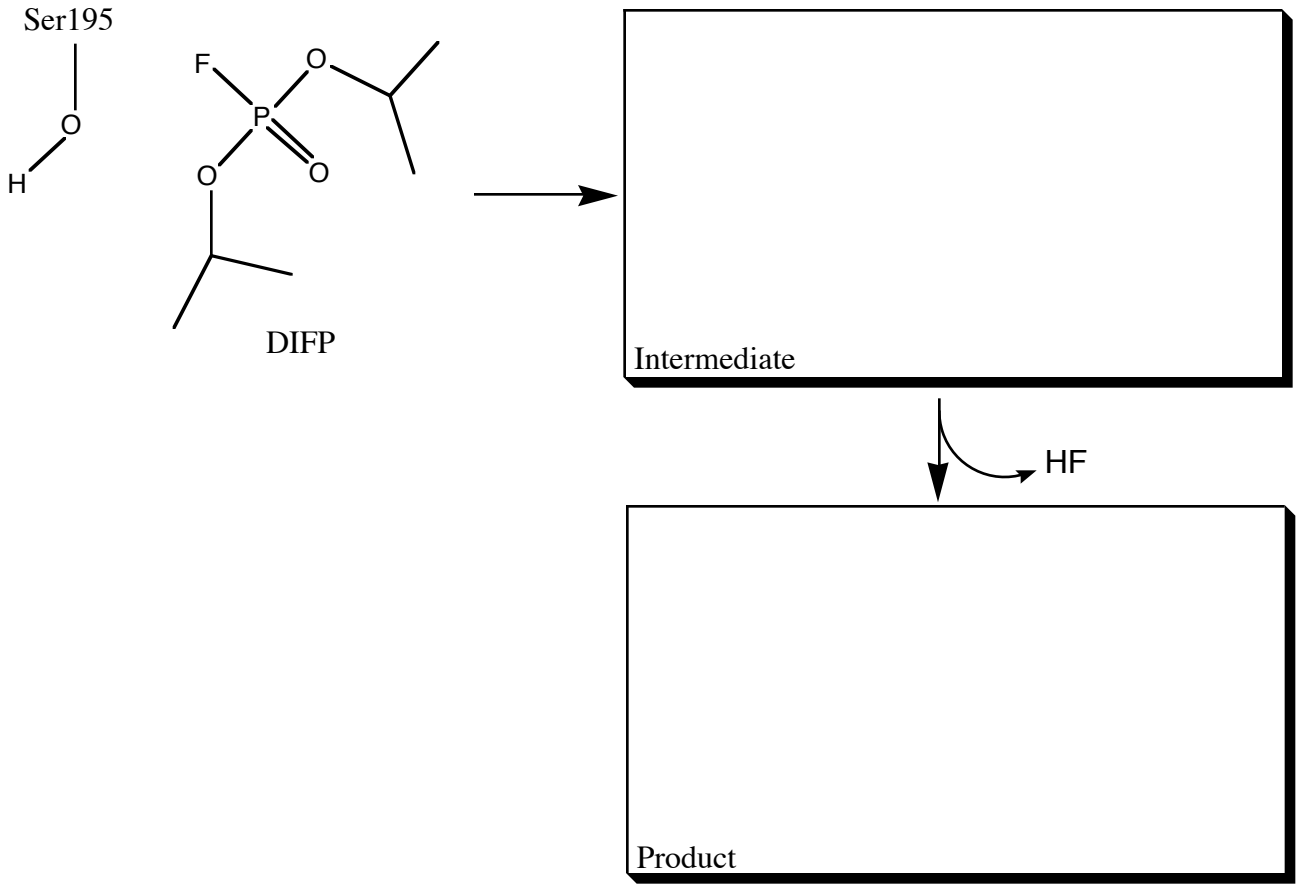
(c; 7 pts) In the box below, fill in the species and equilibria needed to extend the Michaelis-Menten description to include mixed inhibition. Indicate which equilibrium is relevant to pure uncompetitive inhibition, and explain why it is rare to find a pure uncompetitive inhibitor for a simple M-M enzyme.



(d; 6 pts) Why is it not catalytically advantageous to have an extremely stable ES (or EP) complex? What point along the reaction coordinate should be bound most tightly to make an efficient enzyme? Sketch a free energy reaction coordinate diagram to illustrate.

3. (20 points) Enzymatic Reaction Mechanisms.

(a; 10 pts) Di-isopropylfluorophosphate (DIFP) is a potent irreversible inhibitor of serine proteases. The phosphorus atom is extremely electrophilic due to the presence of the electronegative fluorine, which is also a good leaving group. Complete the mechanism below for the reaction between Ser 195 and DIFP. Explain why DIFP does not react with other serines on the protein. (You can answer the last part even if you were unable to draw the mechanism).



(b; 6 pts) Serine proteases still catalyze peptide hydrolysis (slowly) when Ser is replaced by Ala!

1. What principle of enzymatic catalysis explains this observation?
2. Explain why replacement of the other two residues of the catalytic triad with alanine does not further reduce the reaction rate.
3. What class of artificial enzymes work according to the same principle?

(c; 4 pts) What do cofactors do in general? Name a cofactor and describe what kind of reaction it is used for.

4. (15 points) Regulation of Enzyme Activity.

(a; 9 pts) By analogy to cases mentioned in lecture, guess what the main kind of regulation used to modulate each of the following biological processes is, and state why:

1. Changes in the activity of threonine deaminase in response to leucine levels, where leucine is the downstream product of the reaction catalyzed by the enzyme.
2. The response of signal transduction intermediates to the hormone epinephrine, which stimulates glycogen breakdown.
3. The development of the *Drosophila* body plan, whereby an embryo is elaborated into a fly.

(b; 4 pts) Why is ATCase activated by ATP and inhibited by CTP? What kind of inhibition is this?

(c; 2 pts) Why are serine proteases synthesized as inactive zymogens, which must be proteolyzed to become active?

5. (20 points) Carbohydrates and Lipids.

(a; 8 pts) Name three functions for carbohydrates in biochemistry, and the characteristics of carbohydrates which make them especially suited for each of these functions.

(b; 5 pts) When single-tail detergent concentrations exceed the critical micelle concentration, added detergent makes more micelles, not bigger micelles. In contrast, double-tail phospholipid forms bilayers which tend to get larger and larger. Explain these observations.

(d; 7 pts) Briefly describe the experiments which show that transverse diffusion of phospholipids in membranes is slow and that lateral diffusion is fast.

Do Not Write Below This Line

Score:

Question 1: _____ out of 20

Question 2: _____ out of 20

Question 3: _____ out of 25

Question 4: _____ out of 15

Question 5: _____ out of 20

Total: _____ **out of 100**